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硕 士 学 位 论 文

^{99m}Tc 标记 TSPO 新型配体（CB86）在肺癌与炎症中的显像研究

Studies of Imagings in Lung Cancer and Inflammation by ^{99m}Tc Labled New Ligand（CB86）of TSPO

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摘 要

研究背景和目的

肿瘤曾经被定义为一种和心血管病、糖尿病或者慢性呼吸系统疾病等相似的非传染性疾病。尽管它们被认为是随着患者年龄的增大、都市化的不断发展以及不健康的生活习惯而逐渐出现的，人们也慢慢认识到炎症也是导致癌症发病的重要环节之一。很多研究已经证实，炎症是一个明确可以导致肿瘤的危险因子，即伴随着人体内炎症因子的增加，C 反应蛋白水平的升高，肿瘤的危险因素也在增高。炎症的环境可能是导致各种癌症发生的重要原因。如慢性乙肝及丙肝，或肝吸虫病等可增加肝癌风险；又例如空气中的刺激性物质，如石棉，PM2.5 等，可显著增加胸膜间皮瘤或者肺癌的风险。故而炎症的发生发展和肿瘤的一些致病因素密切相关。

而近几十年来，肺癌的发病率与死亡率在世界上多数国家均呈急速上升趋势，是导致死亡的最常见癌症,特别是在欧美某些国家和我国大城市中。肺癌患者大多数为男性, 男女之比为 3：1 ～5：1, 但近年来女性肺癌的发病率也呈明显增加趋势,肺癌已成为全球肿瘤相关性死亡的首要原因。故本文主要选取肺癌与炎症来进行初步研究。在肺癌方面，目前治疗其最佳方法为手术, 但手术后的生存率仍较低，特别是随着人口老龄化速度的加快和生存环境的改变，肺癌对人口的发病和致死率的影响日益显现。早期发现、早期诊断、正确分期和合理治疗对于延长肺癌患者的寿命、改善其生存质量十分重要。因此，寻找新的药物靶标，开发新的诊断肿瘤及抗癌药物具有极其深远的作用。然而现在世界上很多肿瘤和炎症的检出主要依赖于传统影像学，但是传统影像学对其显像却受到各种条件的限制，而且在大多数情况下二者的显像结果比较相似，从而造成了混淆。这也要求找到一种可以将肿瘤和炎症区分开来的新探针，其对于临床上鉴别两者同样具有深远的意义。TSPO（转位蛋白，18kDa）被称为外周苯二氮卓类受体，主要分布在细胞的线粒体外膜中，在很多肿瘤细胞和炎症细胞中均具有高表达。在 TSPO 配体方面，除了 PK 11195，Ro5-4864 等是其经典配体但具有局限性之外，CB86 作为其新型配体近年来愈发受人重视。因 CB86 可以特异性结合细胞中的 TSPO

蛋白，其亲和力约为 PK11195 的 10 倍，并且可以利用较遍及的放射性核素 ^{99m}Tc 标记 CB86 从而在较便宜、普及的 SPECT/CT 下对肿瘤及炎症模型进行显像，故 CB86 被认为是一种具有很大前景且可以显像的前体。因肺癌已居全球各类肿瘤恶性程度的首位，故本文主要研究 TSPO 在肺癌细胞与炎症细胞上的表达及 ^{99m}Tc 标记其配体 CB86 在肺癌模型与炎症模型显像中的初步区别。

实验方法

首先通过 Western blot、流式细胞术观察 TSPO 抗体与正常支气管上皮细胞 16HBe、肺癌细胞 A549、NCI-H446 的结合能力及在这些细胞中 TSPO 蛋白表达量的情况；细胞免疫荧光及化学染色实验检测 TSPO 蛋白的特异性及其在细胞质中的定位；免疫组织化学实验检测蛋白的特异性及其在肺癌组织上的表达情况；Micro-SPECT/CT 显像实验观察该探针在荷瘤裸鼠中的显像效果；脂水分配系数实验检测所标记化合物是否具有脂溶性或是水溶性；体外稳定性检测所标记化合物在生理盐水及小鼠血清中的稳定性；细胞摄取与释放实验检测该探针与细胞中蛋白的结合及外排情况；炎症小鼠模型生物体内分布实验检测探针在体内各器官的摄取与滞留情况；Micro-SPECT/CT 显像实验观察该探针在炎症小鼠中的显像效果。

实验结果

Western blot、流式细胞术结果显示 TSPO 蛋白在正常支气管上皮细胞和不同肺癌细胞株中均表达，但表达量不同。细胞免疫荧光及免疫化学染色实验表明 TSPO 定位在细胞质。脂水分配实验结果表明 ^{99m}Tc-CB86 是水溶性的。体外稳定性实验表明，该标记物在小鼠血清和生理盐水中放置 4 h 后依然没有放射性分解，证明体外稳定性很好。细胞摄取和释放实验说明，其在巨噬细胞中特异性结合很高，并且在 4h 后缓慢从细胞中排出。生物分布实验数据发现它对巨噬细胞具有特异性摄取和良好的滞留能力。对裸鼠肿瘤模型和小鼠炎症模型进行 Micro-SPECT/CT 显像研究，可以看出该标记物在肿瘤模型上不摄取而在炎症上摄取清晰可见。

结论

1. TSPO 抗体可以结合正常肺细胞、肺癌细胞及组织以及巨噬细胞上 TSPO

蛋白，且这些细胞（正常肺细胞除外）上高表达 TSPO。

2.我们成功制备了 ^{99m}Tc-CB86 分子探针，^{99m}Tc-CB86 的标记方法简单可行，标记率较高，稳定性较好，具有良好的 TSPO 蛋白靶向性及特异性，是一个具有前景的新型炎症诊断的显像剂。

关键词：TSPO；肺癌细胞；炎症；^{99m}Tc-CB86

Abstract

Background and Purpose

Tumor is defined once as a noncommunicable diseases like cardiovascular disease, diabetes or chronic respiratory diseases. Although they are considered as the augment of age, urbanization and unhealthy habits,then tumor gradually emerges, people gradually recognized that with the augment of inflammatory factors in the human body and with the elevation of C-reactive protein levels, inflammation is also one of important factors of cancer.Many studies have demonstrated that inflammation is a clear risk factor that can lead to cancer. The environment of Inflammation may be an important which can cause cancer. For example, chronic hepatitis B , hepatitis C or liver fluke disease can increase the risk of liver cancer. Another example is the air of irritating substances, such as asbestos, PM2.5 can significantly increase the risk of lung cancer or mesothelioma. Therefore the development of inflammation is closely related to a number of risk factors of tumor.

In recent decades, the incidence and mortality of lung cancer showed a trend of rapid rise in most countries of the world, it is the leading cause of death in large amounts of common types of cancer, especially in some big cities in China and other countries in Europe and the United States, most of the patients with lung cancer are men, the ratio between men and women is 3:1 ~ 5:1, but in recent years the incidence of female patients with lung cancer also showed an increasing trend significantly, and lung cancer has become the leading cause of cancer death in the world so far. So this article selected lung cancer and inflammation to study preliminarily.In the aspect of lung cancer,the best way to treat with it is surgery, but after the surgery the survival rates are still low. Especially with the change in the acceleration of population aging and the development of living environment, the impact of lung cancer incidence and mortality to population becomes increasingly obvious. So it is very important to

improve the quality of life and prolong the life of the lung cancer patients with early detection, early diagnosis, accurate staging and reasonable treatment. Therefore, searching for new drug targets and the development of new anti-cancer drugs can have far-reaching effects. Now a large amount of detection of tumor and inflammation in the world mainly depends on traditional imaging. However, traditional imaging is limited by a variety of conditions. And in most cases, the imaging results of tumor and inflammation are similar, thus caused confusion for doctors. It also calls for a new probe which can distinguish tumor from inflammation, it also has profound significance to identify them in clinical practice. TSPO (translocator protein, 18 kDa) , known as peripheral benzodiazepine receptors, is mainly distributed in the mitochondrial outer membrane of cells, and it has high expression in both many tumor cells and inflammatory cells. In terms of TSPO ligand, besides PK 11195, Ro5-4864 and so on, which are classic ligands but have their own limitations, CB86 ,as new ligand of TSPO, attracts more and more attention in recent years. Because CB86 can specifically bind to TSPO which is expressed in some kinds of cells and radionuclide ^{99m}Tc which is pervade can labeled CB86,and its affinity is about 10 times as PK11195's. so that it can image tumor and inflammation model in SPECT/CT which is cheap and popular. It is thought to be a target for imaging. Because lung cancer ranks first in various kinds of malignant tumors in the world, therefore this article mainly research TSPO expression in lung cancer cells and inflammatory cells and the imaging differences between lung cancer and inflammation model with its ligand CB86.

Methods

Firstly Western blotting and flow cytometry were used to observe binding capacity of normal lung cells (16HBe), lung cancer cells(A549 and NCI-H446)with TSPO antibody and expression amounts of TSPO in different cells; immunofluorescence and cytochemistry staining detected TSPO specificity and the location in the cytoplasm; immunohistochemistry was used to detect the specificity of the protein and its expression in lung cancer tissues; Experimental observation probe

Micro-SPECT/CT was used to imaging effects in nude mice beared tumor;The octanol/water partition coefficients detected whether the marked compound is fat-soluble or water-soluble; In vitro stability tested the stability of the marked compound in saline and in the serum of mouse; In the cells, cell uptake and efflux assay tested the combination of the probe with the protein in the cells and the excretion of it; the biodistribution in mice model detected the absorption and retention of the probe in each organ in the body; Experimental observation probe Micro-SPECT/CT was used to imaging effects in inflammatory mice.

Results

The results of Western blotting and flow cytometry showed that the antibody has high affinity in lung cancer cells, and it had different amounts of expression in normal bronchial epithelial cell and various kinds of lung cancer cell lines. Immunochemical staining and immunofluorescence results showed that TSPO was localized in the cytoplasm of cells. The octanol/water partition coefficients showed that the compound ^{99m}Tc-CB86 was water-soluble. In vitro stability experiment showed that the marked compound had no radiolysis in mice serum and physiological saline after 4 h ,so that it indicated in vitro stability is quite good. Cell uptake and efflux assays showed that the marked compound had high specific binding in macrophages and it was expelled from cells. The data of biodistribution found that the marked compound had specific uptake and nice retention. The results of imaging by Micro-SPECT/CT showed the imaging effects of nude mice model bearing tumor and inflammatory mice model.

Conclusions

1. TSPO antibody can combined with TSPO protein which is in lung cancer cells and tissues, normal lung cells, and macrophages.TSPO was highly expressed in these cells(besides normal lung cells).

2. We succeeded in preparation of ^{99m}Tc-CB86.Its methods were simple, feasible and had high labeling rate, nice stability, excellent TSPO targeting and specificity. And it was a new promising imaging agent for diagnosing inflammation.

Key words: TSPO; Lung cancer cells; Inflammation; ^{99m}Tc-CB86

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